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Effects of Plant Growth Regulator S-Y on Diurnal Changes in Photosynthetic Parameters and Yield of *Stevia Rebaudina* Bertoni

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Abstract

Selecting the variety of QingTian 2, the effects of plant growth regulator S-Y(PGR-S-Y) on diurnal changes in photosynthetic parameters and yield of *Stevia rebaudina* Bertoni were studied in the open field. The results indicated that the appropriate concentration of PGR-S-Y (T2) significantly increased the SPAD, the utilization rate of CO₂ and the Photosynthetic rate (Pn) respectively, and restricted the midday depression. The effect on the vegetative growth stage was not significant. It significantly increased the fresh leaf weight, the dry leaf weight and the stevioside content in T2 than CK by 10.24%, 11.09% and 4.74% respectively. But when the PGR-S-Y content was over high (T3), the utilization rate of CO₂, the photosynthetic rate (Pn) and photosynthate decreased. At the same time, the vegetative growth stage shortened seriously, the yield and the stevioside content decreased significantly.

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Keywords: Plant growth regulator S-Y; *Stevia rebaudina* Bertoni; diurnal changes in photosynthetic parameters; yield

1. Introduction

Recently, as the increasing requirement of healthy diet, the further studies of components of Stevioside and its pharmacological effects [1-2], the demanded quantity of Stevioside is rising. The *Stevia rebaudina* Bertoni has been introduced and cultivated all over the world and the area of *Stevia rebaudina* Bertoni cultivation is growing, The research reports are becoming more and more, but these mainly concentrate on the morphologic description of new varieties[3], the breeding technologies, the tissue

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extracting methods of stevioside[5-6], the function testing of different proteins, chromosome and genes[7-9] and its habits of growth[10]. As the development of science and technology and the improvement of agricultural productive technology, using the plant growth regulators to regulate the growth and development has become an important measure in the agricultural cultivation [11-12]. But the studying about the effects of plant growth regulators on *Stevia rebaudiana* Bertoni is still lack, and the present work in this study was to research the effects of plant growth regulator S-Y(PGR-S-Y) on diurnal changes in photosynthetic parameters and yield of *Stevia rebaudiana* Bertoni in Chinese region.

2. Materials and methods

The study was carried out in field experimental plot of Qingdao Agricultural University, in Shandong Province, located in Chengyang District, Qingdao City (36° 15'N, 120°20' E). The experimental plot belongs to temperate and monsoonal climate. The annual mean temperature is 12.6°C and the average frost-free date is 179 days over the past years. The soil at the experimental site is loam texture and the plow soil layer of 0-20cm contains 1.35% of total organic matter, 0.09% of total nitrogen, 105.42 mg·kg⁻¹ of available nitrogen, 24.58 mg·kg⁻¹ of available phosphate and 85.72 mg·kg⁻¹ of available potassium.

The variety of *Stevia rebaudiana* Bertoni was No.1. The PGR-S-Y was mixed in the Dryland Technology key laboratory of Shandong Province in Qingdao Agricultural University and was sprayed it. There are 4 treatments in this study with three replications--Control (CK, sweet water), Treatment1 (T1, 50 mg/L), Treatment2 (T2, 100 mg/L) and Treatment3 (T3, 200 mg/L). All plots were randomly arranged, and the area of the plot was 20m² with 3kg compound fertilizer (N:P:K=15%:15%:20%) applied. The *Stevia rebaudiana* Bertoni was treated with the PGR-S-Y in May 18th, 2009 and June 3rd, 2009. The photosynthetic parameters and the SPAD of chlorophyll were measured in the June 24th, 2009. The *Stevia rebaudiana* Bertoni was harvested in the initial flowering stage, and then the yield index was measured.

The photosynthetic parameters of leaves in *Stevia rebaudiana* Bertoni were measured with LI-6400 portable photosynthesis system (LI-COR Biosciences, US), under the condition of open gas road, and the concentration of CO₂ was about 365 μmol/mol. Five plants were measured repetitively and three healthy functional leaves were randomly selected to measure experiment parameters per hour from 6:00 to 18:00 at the same position then got the average as the final result of each time. All the measurement parameters were net photosynthetic rate (P_n, μmol CO₂·m⁻²·s⁻¹), transpiration rate (Tr, mmol H₂O·m⁻²·s⁻¹), leaf to air vapor pressure deficient (V_{pd}, kPa), leaf temperature (T_{leaf}, °C), photosynthetically active radiation (PAR, μmol·mol⁻¹), intercellular CO₂ content (C_i, μmol·mol⁻¹), air CO₂ content (C_a, μmol·mol⁻¹), stomatal conductance (C_s, mmol H₂O·m⁻²·s⁻¹), air relative humidity (RH, %). In this study, we analyzed the meaningful parameters including P_n, G_r and C_i. Measured chlorophyll concentration with SPAD502 (Top Instrument Company Zhejiang, China).

The leaves and stems were separated from the *Stevia rebaudiana* Bertoni in all treatments after harvest in the initial flowering stage. After measuring the fresh leaf weight, the fresh leaves were deactivated the enzymes at 105°C and dried at 50°C. Following which, the dry leaf weight was measured. The stevioside was extracted with the hot-water.

All the data was analyzed with EXCEL and DPS.

3. 3 Results and analysis

3.1. The effect of PGR-S-Y on SPAD of chlorophyll in *Stevia rebaudiana* Bertoni leaves

The effect of PGR-S-Y on the SPAD of chlorophyll in *Stevia rebaudiana* Bertoni was showed in the Fig.1. The tendency of SPAD was $T2 > T1 > CK \geq T3$ overall, the SPAD of *Stevia rebaudiana* Bertoni in T2 was 6.28% higher than that in CK, and it was 2.81% higher in T2 than that in CK, those were both significant, but the diversity of the SPAD of chlorophyll between T1 and T2 were not significant, and there was no significant difference between T3 and CK. It indicated that the proper concentration of PGR-S-Y(T2,T1) could improve SPAD of chlorophyll in *Stevia rebaudiana* Bertoni, and when the concentration was too high (T3). The effect of PGR-S-Y on the SPAD of chlorophyll in *Stevia rebaudiana* was not significant.

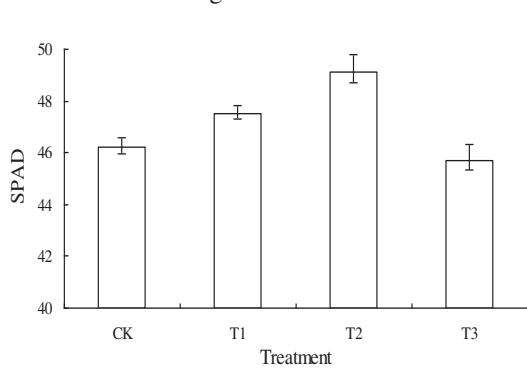


Fig. 1 The SPAD of chlorophyll in different treatment

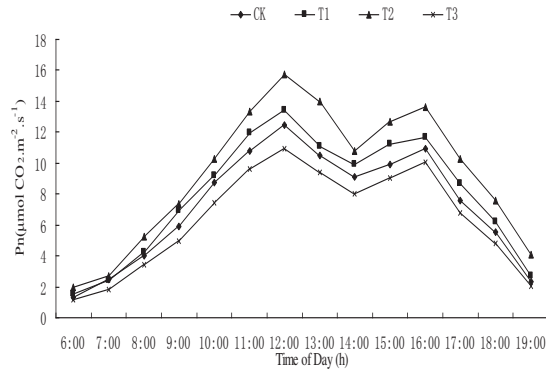


Fig.2 Diurnal changes of net photosynthetic rate(Pn)

3.2. The effect of PGR-S-Y on Pn in *Stevia rebaudiana* Bertoni

The diurnal changes of Pn in *Stevia rebaudiana* Bertoni was presented in Fig.2, it was lower in the morning and in the evening, but higher at about 12:00 pm. It showed double peak obviously, indicated that the *Stevia rebaudiana* Bertoni had significant phenomenon of the midday depression of photosynthesis.

The change of Pn in this study was $T2 > T1 > CK > T3$, indicated that the Pn increased as the rising of concentration of PGR-S-Y in the appropriate range (T1, T2), the too high concentration would decrease the Pn(T3). As well, adequate concentration of PGP-S-Y could relief the extent of the midday depression of photosynthesis. It indicated the adequate concentration of PGP-S-Y could defer the reduction of Pn in the adverse environment. It was worth exploring the cultivation measures, with which the Midday Depression of Photosynthesis would be alleviated to get more photosynthate for the emerging sugar-yielding crops.

3.3. The effect of PGR-S-Y on Gs in *Stevia rebaudiana* Bertoni

The Fig.3 represented that the diurnal change of the Gs was a double-peak trend. The peaks appeared about at 12:00am and 16:00pm respectively, and the former was higher than the latter. The diurnal change in Gs indicated that Gs became higher and higher in the morning as the increase of the temperature and solar radiation, and Gs reduced as the decrease of relative humidity in the field after midday. The reduction of the Gs would cause the plants of the *Stevia rebaudiana* Bertoni to decrease the temperature and to reduce the transpiration in vivo.

In the proper range, the Gs enhanced with the increase of the concentration of the PGR-S-Y(T1,T2),

and weakened when the concentration was over high (T3). Some relative researches indicated that the potassiumion was the main reason to adjust the opening and closing of the stomatas in the leaf, so the appropriate application of the PGR-S-Y could promote *Stevia rebaudiana* Bertoni to absorb and balance the nutrient elements.

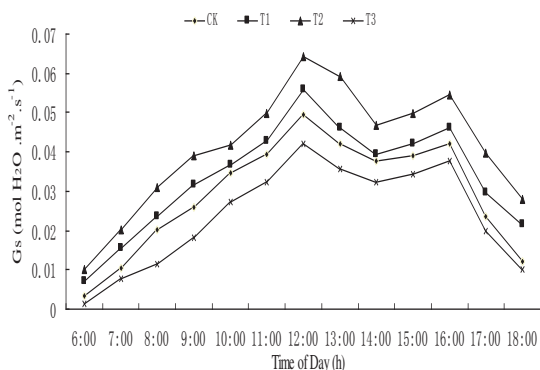


Fig.3 The diurnal changes of stomatal conductance (GS)

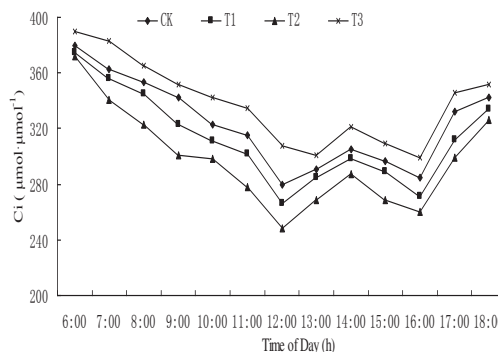


Fig.4 The diurnal changes of intercellular CO₂ content (Ci)

3.4. The effect of PGR-S-Y on Ci in *Stevia rebaudiana* Bertoni

The diurnal changes of Ci showed a single-peak curve in all treatments, and the tendency was opposite to that in Pn (Fig.4). The Ci was higher in the morning and in the evening, and the Ci was at its bottom at about 12:00 pm, became increasing after the noon and went back to its bottom again. It also meant that the Ci increased as "midday depression photosynthesis" occurred at noon. It indicated that the time of the double troughs was corresponded to the time of the double peaks of Pn and Gs. The Ci decreased because more CO₂ was fixated by the chloroplast to produce Carbohydrate when photosynthetic rate (Pn) was at a high level.

The different concentration of PGR-S-Y has significant effect on the utilization ratio of CO₂ in *Stevia rebaudiana* Bertoni, and there was positive correlation between the concentration of PGR-S-Y in proper range and the utilization ratio of CO₂, it was highest in the treatment of T2, and was conspicuously higher than that in other treatments. But when the concentration was over high, the utilization ratio of CO₂ significantly decreased (T3).

3.5. The effect of PGR-S-Y on the yield of the *Stivia rebaudiana* Bertoni

As the Table.1 showed, the florescence of *Stivia rebaudiana* Bertoni advanced and the vegetative growth stage shortened seriously in T3, and it was not significant in other treatments. The leaf fresh weigh, leaf dry weight and the yield and the stevioside content were 10.24%, 11.09% and 4.74% higher in T2 than that in CK respectively, and the increase reached extremely significant level. The leaf fresh weigh, leaf dry weight, the yield and the stevioside content in T1 were significantly higher than that in CK. But the leaf fresh weigh, leaf dry weight, the yield and the stevioside content of T3 were 67.63%, 84.76% and 6.77% lower than that in CK respectively. It indicated that there was no change at the vegetative growth stage. The leaf fresh weigh, leaf dry weight, the yield and the stevioside content were significantly increased as the increasing of the concentration of PGR-S-Y in appropriate range (T1, T2). But when the

concentration was too high, the vegetative growth stage, the yield and the stevioside content decreased seriously (T3).

Table.1 The vegetative growth stage, yield and stevioside contention

| Treatment | Vegetative Growth Stage(d) | Leaf Fresh Weight(g/plant) | Leaf Dry Weight (g/plant) | Leaf Dry Weight (kg/666.67m ²) | Stevioside Content (%) |
|-----------|----------------------------|----------------------------|---------------------------|--|------------------------|
| CK | 79 | 142.12cB | 28.49cB | 170.94 | 12.46cB |
| T1 | 78 | 148.23bB | 29.84bB | 179.04 | 12.76bAB |
| T2 | 79 | 156.67aA | 31.65aA | 189.90 | 12.93aA |
| T3 | 61 | 84.78dC | 15.42dD | 92.52 | 11.67dC |

4. Discussion and conclusion

Previous studies on the photosynthesis of crops indicated that the yield of the crops depended mainly on the photosynthetic area, the photosynthetic rate, the photosynthetic time and the distribution and translation of the photosynthates. It was a law there was positive correlation not negative correlation between the photosynthetic rate and the crop yield, but the law performed only on the condition that other excellent physiological function and economic characters combined with various stress resistance [13]. In this study, the improvement of yield and stevioside content in T2 was significant, because the vegetative growth stage was enough long and the Pn in T2 was the highest of all treatments. The results in this study coincided with the former studies.

The appropriate application of the PGR-S-Y could promote to absorb and balance the nutrient elements in *Stivia rebaudiana* Bertoni, raise the Gr, provide enough conditions and materials for the photosynthesis, improve the photosynthates, and provide material condition for improving the yield. And proper erogenous supplement of PGR-S-Y decreased the endogenous synthesis of PGR-S-Y in the leaves, and made the joint biosynthesis precursor (Geranylgeranyl Pyrophosphate GGPP) of S-Y, chlorophyll and stevioside more [14-15], so it could improve the content of chlorophyll, provide more essential condition for photosynthesis. At the same time, it could promote the synthesis of the stevioside, increase the content of stevioside in the plant.

The results in this study indicated that the appropriate treatment of PGR-S-Y (T2) increased the SPAD significantly, the utilization rate of CO₂ and the Photosynthetic rate, and alleviated the midday depression. It had no effect on the vegetative growth stage, and it significantly increased the yield and the stevioside content for 11.09% and 4.74% respectively than CK. But when the PGR-S-Y content was too high(T3), it decreased the utilization rate of CO₂, the Photosynthetic rate, and photosynthate. At the same time, the vegetative growth stage shortened seriously, also the yield and the stevioside content decreased significantly. So this study could provided the technical guidance for the cultivation of *Stivia rebaudiana* Bertoni..

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